REMARKS/ARGUMENTS

The application has been amended. In particular, paragraph [0002] has been amended to correct the priority claim. Claim 28 has been amended in order to better define the method of the present invention. Claim 29 has been amended to correct a minor typographical error. Also, claims 30 and 34 have been canceled, and claims 35-37 have been added. Support for these amendments can be found in the application as filed.

Claim Rejections Under 35 U.S.C.§112, first paragraph

Written Description Rejection

The Examiner has rejected claims 16-18 and 28-34 under 35 U.S.C.§112, first paragraph, alleging that the subject matter was not sufficiently described in the specification. In particular, with respect to claim 28, the Examiner is of the opinion that there is no statement or suggestion in the specification that protein/RNA interactions can be determined when the protein is labeled with an acceptor dye, while at the same time the RNA is labeled with a donor dye.

As the Examiner may be aware, according to the Written Description Guidelines, the analysis as to whether Applicants had possession of the claimed invention must be conducted from the standpoint of one of ordinary skill in the art at the time the application was filed. Moreover, information which is well known in the art does not have to be described in detail in the specification according to these Guidelines. Applicants submit that it was well known in the art at the time the application was filed that protein/RNA interactions can be determined regardless of whether (i) the donor is attached to the protein, and the acceptor is attached to the RNA, or (ii) the donor is attached to the RNA, and the acceptor is attached to the protein. In fact, inventors Tamilarasu and Rana are co-authors of the cited Zhang, et al. publication, where protein/RNA interactions were determined under situation (ii) above. Thus, not only was this situation (ii) known in the art, it was clearly delineated in this publication and one of ordinary skill in the art recognizes that FRET occurs in either situation (i) and (ii) above. For these

reasons, the application satisfies the written description requirement under Section 112, first paragraph, and the rejection should properly be withdrawn.

Moreover, Applicants have provided a working example where the protein is labeled with a first fluorescent dye molecule, and the RNA is labeled with a second fluorescent dye molecule that is capable of participating in fluorescence resonance energy transfer with the first dye molecule. As described in paragraph [0033], the methods described in the application are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Applicants submit that both situation (i) and situation (ii) above are encompassed within the spirit of the invention.

In view of these facts, Applicants request withdrawal of these rejections.

Enablement Rejection

The Examiner has also rejected claims 16-18 and 28-34 under 35 U.S.C. §112, first paragraph for containing subject matter which is allegedly not enabled. With respect to claim 28, the Examiner stipulates that the skilled artisan would be able to determine the distance between two fluorophores. However, he is of the opinion that the specification does not teach the skilled artisan how to determine the proximity between the entire protein molecule and the entire RNA molecule. Also, with respect to claim 30, he alleges that the specification provides no guidance as to how to determine the degree of binding between a protein and an RNA molecule, and does not teach a skilled artisan how to determine a dissociation contant which is used to quantitate this binding.

Applicants have amended claim 28 in accordance with the Examiner's suggestions. In particular, step (g) now involves determining the proximity between the dye molecules.

The rejection of claim 30, now canceled, will be addressed with respect to new claims 36 and 37. Applicants do not agree at all with the Examiner's allegations that the specification

provides no guidance as to how to determine the degree of binding between a protein and an RNA molecule, nor with his allegations that the specification does not teach a skilled artisan how to determine a dissociation contant which is used to quantitate this binding. For example, with reference to Figures 2A and 2B and the description in paragraph [0031] on pages 13-14, Applicants disclose that the binding affinity between a Tat peptide and TAR RNA can be determined by examining the fluorescence quenching of Tat-fluorescein at several different TAR-Rhodamine concentrations. Applicants also disclose that the dissociation constant of the RNA and Tat peptide can be obtained by fitting data to quadratic equation (1), in accordance with Muller, et al. (1991) Biochemistry 30, 3709-3715. By fitting the data to this quadratic equation, Applicants' results showed that Tat-Fluorescein binds to the TAR-Rhodamine with a dissociation constant (K_D) of 1.0 ± 0.5 nM. Therefore, Applicants submit that the subject matter of new claims 36 and 37 is fully enabled, and that sufficient guidance has been given as to how to determine the binding affinity between the protein and the RNA.

In view of the amendments and the arguments presented herewith, Applicants respectfully request withdrawal of these rejections.

Claim Rejections Under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 15 and 29 under 35 U.S.C. §112, second paragraph.

The rejection of claim 15 will be addressed with respect to claim 28, since claim 15 was previously canceled in Applicants' Response filed September 8, 2004. Claim 28 has been amended in accordance with the Examiner's suggestions. In particular, step (g) now includes determining the proximity between the dye molecules. The rejection of claim 29 has also been addressed in the claim amendments presented herewith.

Claim Rejections Under 35 U.S.C. §103 Over U.S. Patent No. 6,316,194 to Karn, et al.

The Examiner has rejected claims 16, 18 and 28-31 under 35 U.S.C. §103 as allegedly being unpatentable over U.S. Patent No. 6,316,194 to Karn, et al. (hereinafter the '194 patent).

This rejection is respectfully traversed on the basis that this reference coupled with common knowledge fails to set forth a *prima facie* case of obviousness.

The method of claim 28 now recites "synthesizing a site specific modified protein". As disclosed in the Application, production of the site specific modified protein involves replacing an amino acid of the protein, other than lysine or cysteine, at a desired location of labeling with an amino acid analog. Amended claim 28 also recites that site-specific labeling of the modified protein occurs at the site of the amino acid analog. Moreover, claim 28 now recites that labeling is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine. Support for these amendments can be found in the application, at paragraphs [0023], [0027] and [0028], for example.

The '194 patent discloses the use of antimicrobial compounds, such as peptides, that already contain functional groups that are appropriate for the introduction of fluorescent dyes. This is different from the present invention as defined in claim 28 where the protein is engineered (i.e., synthesized) to contain an amino acid analog for site-specific labeling thereof. The incorporated amino acid analog includes a functional group, such as an acetyl group or a formyl group, to which a fluorescent dye molecule is conjugated, as recited in new claim 35 (see, for example, page 6, line 5).

Also, the '194 patent is different from the present invention in that it fails to disclose or suggest selectively modifying a single, unique amino acid residue in a protein/peptide with a reporter molecule (i.e., label) without causing any substantial effect on other functional groups, as would be required for site-specific labeling. In particular, it is commonly understood in the art that site-specific modification of proteins and peptides with reporter molecules involves covalent derivatization of a functional group of a single, unique amino acid residue in the protein without causing any substantial effect on other functional groups, as described in paragraph [0003] of the application.

Furthermore, the present claims stipulate that the site-specific labeling of the amino acid analog is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine. This feature is neither disclosed nor suggested in the '194 patent. As will be described in further detail below, this inventive feature presents significant advantages.

Moreover, the '194 patent, at best, provides a general teaching to conjugate a fluorescent dye to a laundry list of functional groups, which may be contained in antimicrobial molecules. However, the '194 patent fails to disclose conjugating the fluorescent dye to a peptide containing anything other than possibly amine or dehydroalanine functional groups. For example, only Table 1 and Table 4 appear to disclose peptide antimicrobials, and these contain amine or dehydroalanine groups, respectively. Such limited disclosure clearly does not teach or suggest the subject matter as claimed.

In fact, that which is disclosed in the '194 patent would lead the skilled artisan in a direction which is not claimed, since it suggests reacting various dye derivatives with primary and secondary amines, such as lysine *epsilon* amino groups. In contrast, the method of the present invention requires replacement of an amino acid other than lysine or cysteine with an amino acid analog which is then site-specifically labeled. It is a well accepted principle that references which teach away cannot form the basis of an obviousness rejection.

In summary, the '194 patent relied upon coupled with the alleged common knowledge that an amino acid at a given position in a protein can be modified, fails to produce the claimed invention. In view of the amendments and remarks presented herewith, Applicants respectfully request withdrawal of these rejections.

Claim Rejections Under 35 U.S.C. §103 over U.S. Patent No. 6,573,045 to Karn, et al.

The Examiner has also rejected claims 16, 18, 28-31 under 35 U.S.C. §103 as allegedly being unpatentable over U.S. Patent No. 5,573,045 to Karn, et al. (hereinafter, the '045 patent).

In particular, the Examiner states the following with respect to the synthesis of a FAM labeled peptide at column 28, lines 34+:

> **The implication is that the "FAM" group was attached to the N-terminus of the peptide while the protecting groups were still on the side chains; otherwise one would expect reaction at the lysine *epsilon* amino groups. The first point is that when protecting groups are present on the side chains of amino acids, the resulting protein qualifies as a "site specific modified protein".**

This rejection is respectfully traversed on the basis that this reference coupled with common knowledge fails to set forth a *prima facie* case of obviousness.

As mentioned above, claim 28 now recites that site-specific labeling of the modified protein occurs at the site of the amino acid analog. The '045 patent fails to teach disclose or suggest this feature. For example, the Examiner considers that the Fmoc derivatives of Karn qualify as "amino acid analogs". This may or may not be true. However, Applicants submit that the Fmoc derivatives, even if they are amino acid analogs, are not labeled with a fluorescent dye molecule as provided in Applicants' claims. In the '045 patent, the label is placed on an N-terminal amino acid, which is <u>not</u> an amino acid analog. This is different from the present invention where the label is placed on the incorporated amino acid analog.

Furthermore, claim 28 now recites that labeling is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine. In contrast, the '045 patent fails to disclose, teach or suggest that site-specific labeling of the protein/peptide is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine, as provided in Applicants' claims. In fact, as recognized by the Examiner in the above-referenced passage, it suggests quite the opposite. That is to say, the implication is that attachment of the label at the N-terminal end of a peptide containing lysine *epsilon* amino groups

requires some protection of these side chains. This is distinctly different from the present invention, where once the protein has been synthesized, no orthogonal protection of nucleophilic lysine or cysteine side chains is required before the site-specific labeling can occur. Also, since the nucleophilic side chains do not require orthogonal protection in the instant methods, no deprotection reactions are required before the site-specifically labeled protein can be utilized. Therefore, this is a feature of the present invention which provides considerable advantages.

In summary, the '045 patent relied upon coupled with the common knowledge that an amino acid at a given position in a protein can be modified, fails to produce the claimed invention. In view of the amendments and remarks presented herewith, Applicants respectfully request withdrawal of these rejections.

Claim Rejections Under 35 U.S.C. §103 over Zhang, et al.

The Examiner has also rejected claims 16, 18 and 28-31 under 35 U.S.C.§103 as allegedly being unpatentable over Zhang, et al. (J. Biol. Chem. 275, 34314, 2000), hereinafter Zhang, on which the present inventors are co-authors. The Examiner's rejections are on the grounds that Zhang discloses a study of the interactions between TAR RNA and a tat peptide, using FRET, where Fluorescein was bonded to the RNA and Rhodamine to the peptide.

This rejection is respectfully traversed on the basis that this reference coupled with common knowledge fails to set forth a *prima facie* case of obviousness.

Applicants previously argued that Zhang does not disclose replacement of an amino acid with an amino acid analog. However, the Examiner alleges that this is a benign structural change. We also argued that Zhang's incorporation of the label into the protein at a lysine residue was precluded by the claims, and was actually a teaching away from the present invention. In response, the Examiner states that his ground of rejection does not require a structural alteration at a lysine, and alleges that a homolog of almost any amino acid, other than lysine or cysteine, would meet the requirements of the claims.

Applicants wish to remind the Examiner that his decision to maintain or withdraw a rejection requires consideration of all the evidence of record. The totality of the evidence includes any rebuttal evidence an applicant may have submitted. See *In re Eli Lilly & Co.*, 902 F.2d 943, 945, 14 U.S.P.Q.2d 1741, 1743 (Fed. Cir. 1990). After a *prima facie* case of obviousness has been made and rebuttal evidence submitted, all the evidence must be considered anew.

In addition to the above-mentioned differences between Zhang and the present invention, Zhang also fails to teach, disclose or suggest that site-specific labeling is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine. In fact, the implication is that attachment of the label at the Lys-19 *epsilon* amino group in Zhang requires some protection of other nucleophilic side chains. Otherwise, one would expect reaction at the other nucleophilic side chains. As mentioned above, the ability to selectively modify one amino acid, even in the presence of nucleophilic side chains of lysine or cysteine (i.e., unprotected side chains), is a feature of the present invention which provides considerable advantages.

In summary, the Zhang reference relied upon coupled with the common knowledge that an amino acid at a given position in a protein can be modified, fails to produce the claimed invention. In view of the amendments and remarks presented herewith, Applicants respectfully request withdrawal of these rejections.

Claim Rejections Under 35 U.S.C.§103 over Czworkowski, et al. or Odom, et al. (1990) or Odom, et al. (1984)

The Examiner has also rejected claims 16 and 28-31 under 35 U.S.C.§103 as allegedly being unpatentable over Czworkowski, et al. (Biochemistry 30 (19) 4821-30, 1991) or Odom, et al. (Biochemistry 29 (48) 10734-44, 1990) or Odom, et al. (Biochemistry 23, 5069, 1984). In particular, the Examiner states the following:

> **Each of Czworkowski (1991), Odom (1990) and Odom (1984) describe experiments in which RNA and protein are both labeled with a fluorophore, and the extent of fluorescence energy transfer between the two probes is determined. None of the references discloses that the ribosomal protein is site specific[ally] modified". However, the protein chemist of ordinary skill would recognize that extending a side chain by a single methylene group would not adversely affect the function or activity of the ribosomal protein. ...**

This rejection is respectfully traversed on the basis that the cited references relied upon coupled with common knowledge fail to set forth a *prima facie* case of obviousness.

The Examiner admits that these references fail to disclose a site specific modified protein, but couples these references with the common knowledge that an amino acid at a given position in a protein can be modified to arrive at the claimed invention. In response, Applicants submit that this combination does not produce the invention as presently claimed.

In particular, the present invention not only requires synthesis of a site specific modified protein, it also involves subsequent site-specific labeling of the protein at the site of the amino acid analog. In particular, this site-specific labeling requires selectively modifying a single, unique amino acid residue in a protein/peptide with a reporter molecule (i.e., label) without causing any substantial effect on other functional groups, as commonly understood in the art. The claims also stipulate that the site-specific labeling is capable of occurring in the absence of orthogonal protection of nucleophilic side chains of lysine and cysteine. These inventive features are neither disclosed, nor suggested by the cited references.

Therefore, the cited references relied upon coupled with the common knowledge that an amino acid at a given position in a protein can be modified, fail to produce the claimed

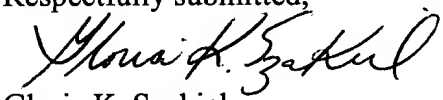
invention. In view of the amendments and remarks presented herewith, Applicants respectfully request withdrawal of these rejections.

Summary

In conclusion, Applicants wish to remind the Examiner that the Court has firmly established that even though the prior art could be modified, the art must still have suggested the modification. Often times, particularly with the aid of hindsight, the art appears modifiable in a manner that will yield the claimed invention. That itself will not make the resultant modification obvious, however. The art must still suggest the desirability of the modification. See *In re Gordon*, 733 F.2d 900, 902, 221, U.S.P.Q. 1125, 1127 (Fed. Cir. 1984).

Applicants submit that the claims, as amended, are patentably distinct and allowable in form. An allowance of the claims is respectfully requested. Should the Examiner have any questions regarding this Response, he is encouraged to contact the undersigned agent at the telephone number set forth below.

Respectfully submitted,



Gloria K. Szakiel
Registration No. 45,149
Agent for Applicant

HOFFMANN & BARON, LLP
6900 Jericho Turnpike
Syosset, NY 11971
(973) 331-1700